

# Unit-2

## Pharmaceutical Biotechnology

### B.Pharma 6<sup>th</sup> Sem Notes

#### Unit: 2

- Study of cloning vectors, restriction endonucleases and DNA ligase.
- Recombinant DNA technology. Application of genetic engineering in medicine.
- Application of r DNA technology and genetic engineering in the production of:
  - i) Interferon
  - ii) Vaccines- hepatitis- B
  - iii) Hormones-Insulin.Brief introduction to PCR.

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### Cloning Vectors:

A cloning vector is a DNA molecule that can carry foreign DNA into a host cell and help it multiply. Think of it as a vehicle that transports genes from one organism to another. The most commonly used cloning vectors are plasmids, which are small, circular DNA molecules found naturally in bacteria.

### OR

Cloning vectors are DNA molecules that can carry foreign DNA fragments into host cells for replication and expression. They are essential tools in genetic engineering and recombinant DNA technology.

### Definition and Purpose

A cloning vector is a small piece of DNA (typically plasmid, phage, or cosmid) that can autonomously replicate within a host cell and is used to transfer foreign genetic material into another cell. The primary purposes include:

- Gene amplification (producing multiple copies)
- Gene expression (producing proteins)
- Gene storage and manipulation
- Creation of recombinant organisms

### Features of Cloning Vectors

- **Origin of Replication (ori):** Allows autonomous replication within the host cell
- **Selectable Marker:** Usually antibiotic resistance genes (e.g., ampicillin, tetracycline resistance) that allow selection of transformed cells
- **Multiple Cloning Site (MCS):** Contains multiple unique restriction enzyme recognition sites for insertion of foreign DNA
- **Small Size:** Typically 2-10 kb for easy manipulation and high transformation efficiency
- **High Copy Number:** Ensures adequate quantities of cloned DNA
- **Reporter Gene:** Such as lacZ for blue-white screening

### Types of Cloning Vectors

#### A. Plasmid Vectors

Plasmids are circular, double-stranded, extra-chromosomal DNA molecules found naturally in bacteria. They are the most commonly used vectors.

- **Size:** 2-10 kb
- **Insert capacity:** Up to 10 kb
- **Examples:** pBR322, pUC18, pUC19
- **Advantages:** Easy to handle, high transformation efficiency, well-characterized

#### B. Bacteriophage Vectors

Bacteriophages (bacterial viruses) can be modified to carry foreign DNA.

- **Lambda ( $\lambda$ ) phage:** Can accommodate 15-20 kb inserts
- **M13 phage:** Used for single-stranded DNA production and DNA sequencing
- **Advantages:** Efficient packaging, larger insert capacity than plasmids



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### C. Cosmid Vectors

Hybrid vectors combining features of plasmids and phage  $\lambda$ .

- **Insert capacity:** 35-45 kb
- **Features:** Contain cos sites from  $\lambda$  phage and plasmid ori
- **Use:** Cloning large DNA fragments, genomic library construction

### D. Bacterial Artificial Chromosomes (BACs)

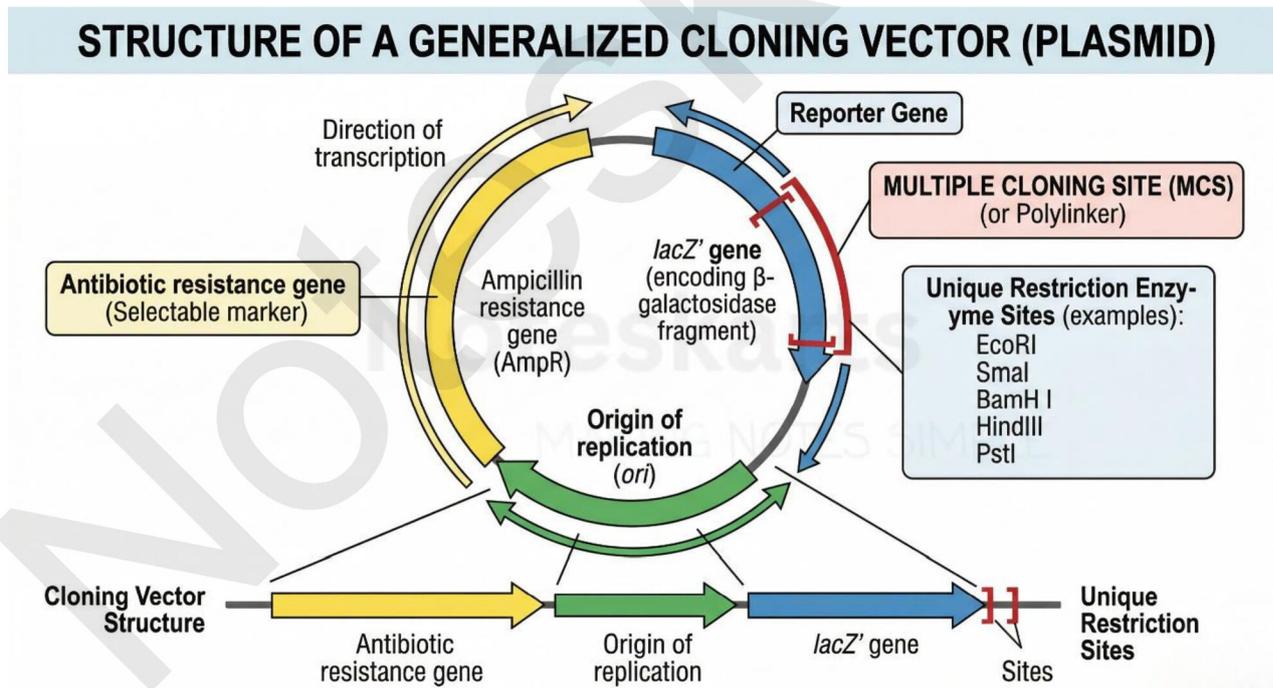
- **Insert capacity:** 100-300 kb
- **Based on:** F plasmid of E. coli
- **Use:** Genome mapping, large gene cloning

### E. Yeast Artificial Chromosomes (YACs)

- **Insert capacity:** 200-2000 kb
- **Features:** Contain telomeres, centromere, and autonomously replicating sequences
- **Use:** Human genome project, cloning very large genes

## Structure of a Cloning Vector

The diagram below shows the essential components of a typical plasmid cloning vector:



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### Restriction Endonucleases

#### Discovery and Nomenclature

Discovered by Werner Arber, Hamilton Smith, and Daniel Nathans (Nobel Prize 1978). These enzymes are part of the bacterial restriction-modification system that protects bacteria from foreign DNA (such as viral DNA).

#### Naming Convention:

- First letter: Genus of organism (italic)
- Next two letters: Species name (italic)
- Letter/number: Strain or serotype
- Roman numeral: Order of discovery

Example: EcoRI = from *Escherichia coli*, strain R, first enzyme discovered

#### Classification of Restriction Enzymes

##### Type I Enzymes

- Cut DNA at random sites far from recognition sequence
- Require ATP and S-adenosylmethionine
- Not useful for gene cloning

##### Type II Enzymes (Most Important for Cloning)

- Cut DNA at specific sites within or near recognition sequence
- Require only  $Mg^{2+}$  ions
- Recognition sequences are usually 4-8 bp palindromes
- Examples: EcoRI, BamHI, PstI, HindIII

##### Type III Enzymes

- Cut DNA 20-25 bp away from recognition site
- Limited use in molecular biology

#### Recognition Sequences and Cutting Patterns

- Type II restriction enzymes recognize palindromic sequences (read the same on both strands in 5' to 3' direction) and produce different types of ends:

##### A. Sticky (Cohesive) Ends

Produce single-stranded overhangs that can base-pair with complementary sequences.

- **EcoRI recognition:** 5'-G↓AATTC-3' (produces 5' overhang)
- **PstI recognition:** 5'-CTGCA↓G-3' (produces 3' overhang)
- **Advantage:** Easy to ligate DNA fragments with complementary ends

#### Molecular Scissors:

Restriction endonucleases are enzymes that cut DNA at specific sequences. They are like molecular scissors that recognize particular DNA sequences and make precise cuts. These enzymes are naturally found in bacteria where they protect against viral infections.

The diagram below shows how restriction enzymes work:



### EcoRI ENDONUCLEASE ACTION: STICKY END PRODUCTION

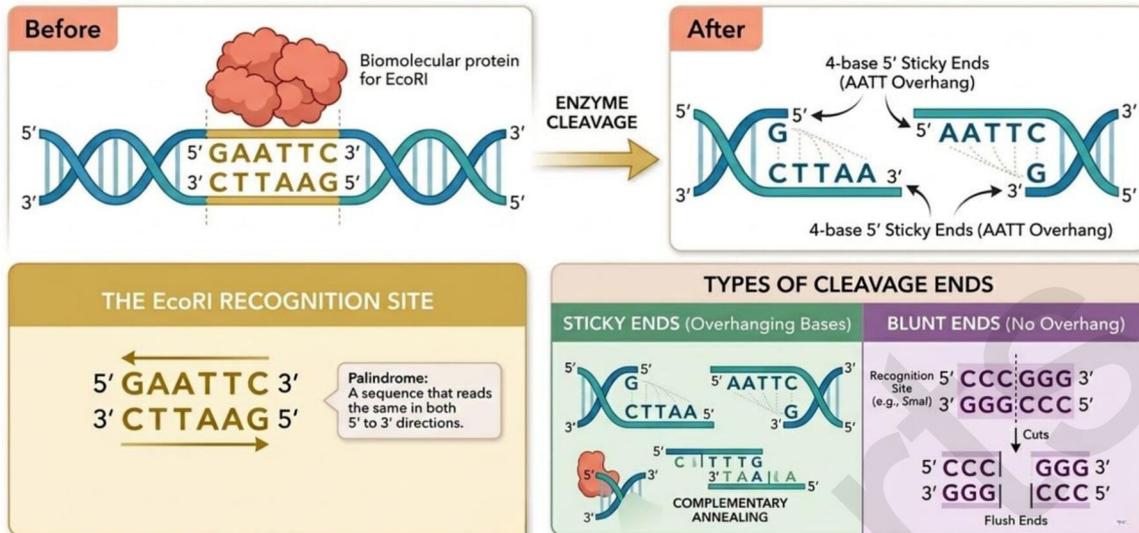


Figure 2: How EcoRI restriction enzyme cuts DNA to create sticky ends

#### Features:

- **Recognition Site:** Each enzyme recognizes a specific palindromic DNA sequence (reads same forward and backward)
- **Sticky Ends:** Staggered cuts create overhanging single-stranded DNA that can easily join with complementary sequences
- **Blunt Ends:** Straight cuts across both strands with no overhang

#### DNA Ligase

DNA ligase is an essential enzyme that catalyzes the formation of phosphodiester bonds between adjacent nucleotides, joining DNA fragments together. It is the molecular glue in genetic engineering.

#### Mechanism of Action

DNA ligase catalyzes the formation of a phosphodiester bond between the 3'-hydroxyl group of one nucleotide and the 5'-phosphate group of another, sealing nicks in the DNA backbone.

#### The reaction occurs in three steps:

1. Adenylation of ligase enzyme (activation)
2. Transfer of AMP to 5'-phosphate of DNA
3. Formation of phosphodiester bond and release of AMP

#### Types of DNA Ligase

##### A. E. coli DNA Ligase

- Uses NAD<sup>+</sup> as cofactor
- Works primarily on sticky ends
- Less efficient with blunt ends

##### B. T4 DNA Ligase (Most Common in Lab)



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- Derived from bacteriophage T4
- Uses ATP as cofactor
- Can ligate both sticky and blunt ends
- Most versatile and widely used

### 3.3 Factors Affecting Ligation Efficiency

- **Temperature:** Usually 16°C for sticky ends, 25°C for blunt ends
- **DNA concentration:** Optimal ratio of insert to vector (typically 3:1 or 5:1)
- **Incubation time:** Longer for blunt ends (overnight) vs sticky ends (1-2 hours)
- **Buffer conditions:** pH, salt concentration, presence of ATP
- **End compatibility:** Sticky ends ligate much more efficiently than blunt ends

### 3.4 Applications

- Joining insert DNA to vector in cloning
- DNA repair in vivo
- DNA replication and recombination
- Next-generation sequencing library preparation
- Site-directed mutagenesis

### How It Works

1. DNA fragments with complementary sticky ends come together through base pairing
2. DNA ligase seals the gaps in the sugar-phosphate backbone
3. The result is a continuous, stable DNA molecule
4. T4 DNA Ligase (most common) can join both sticky and blunt ends



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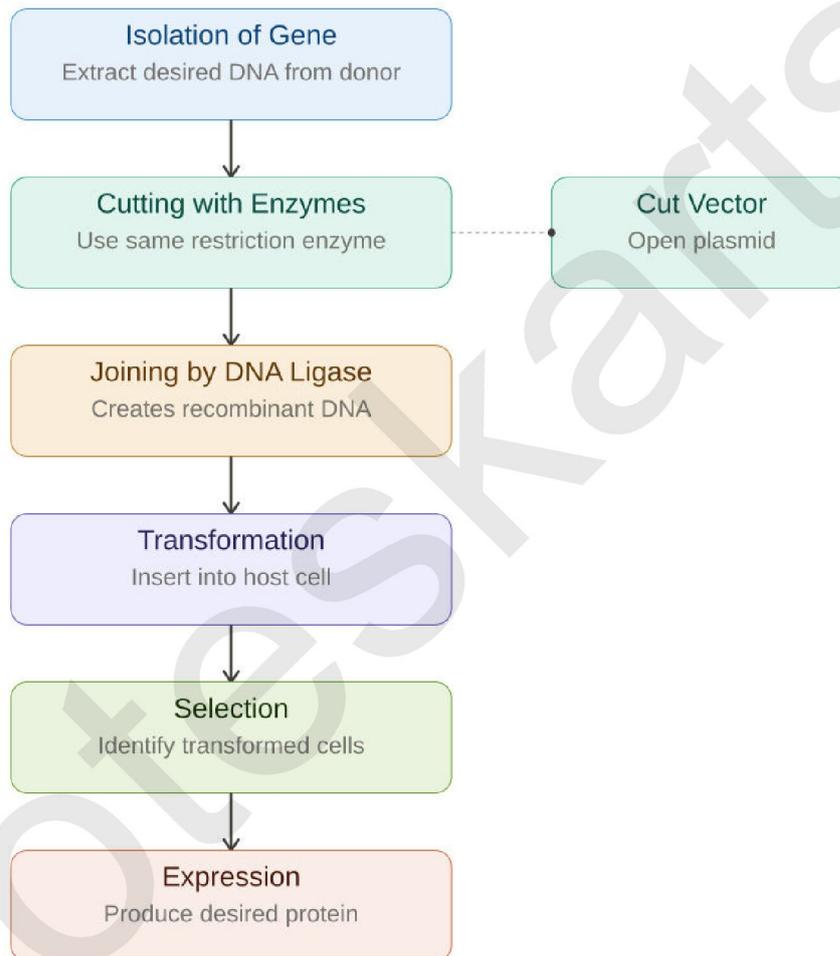
### Recombinant DNA Technology

#### The Complete Process

Recombinant DNA technology combines genetic material from different sources to create new DNA sequences. This process allows scientists to transfer useful genes from one organism to another, enabling the production of important proteins and medicines.

The flowchart below shows all the steps involved:

#### Recombinant DNA Technology Process



*Figure 3: Complete Recombinant DNA Technology Process*

#### Step-by-Step Process

5. **Isolation:** The desired gene is identified and extracted from donor DNA
6. **Cutting:** Both the gene and vector are cut with the same restriction enzyme
7. **Ligation:** DNA ligase joins the foreign gene into the vector
8. **Transformation:** The recombinant DNA is inserted into host cells (usually bacteria)
9. **Selection:** Transformed cells are identified using antibiotic resistance markers
10. **Expression:** Host cells are grown in large quantities to produce the desired protein



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## Applications Of Genetic Engineering In Medicine

- Gene cloning: cutting vector and insert DNA
- DNA fingerprinting and RFLP analysis
- Gene mapping and genome analysis
- Site-directed mutagenesis
- Diagnostic applications

## Production of Interferon

Interferons are proteins that help our immune system fight viral infections and have anti-cancer properties. There are three main types: alpha, beta, and gamma interferons.

**OR**

Interferons are a group of signaling proteins (cytokines) produced by host cells in response to viral infections, tumors, and other pathogens. They are named for their ability to interfere with viral replication.

### Types of Interferons:

- **Interferon- $\alpha$  (Alpha):** Produced by leukocytes
- **Interferon- $\beta$  (Beta):** Produced by fibroblasts
- **Interferon- $\gamma$  (Gamma):** Produced by T-lymphocytes and NK cells

## Functions and Medical Uses

### Functions:

- Antiviral activity (inhibit viral replication)
- Immune system modulation
- Anti-tumor effects
- Cell growth regulation

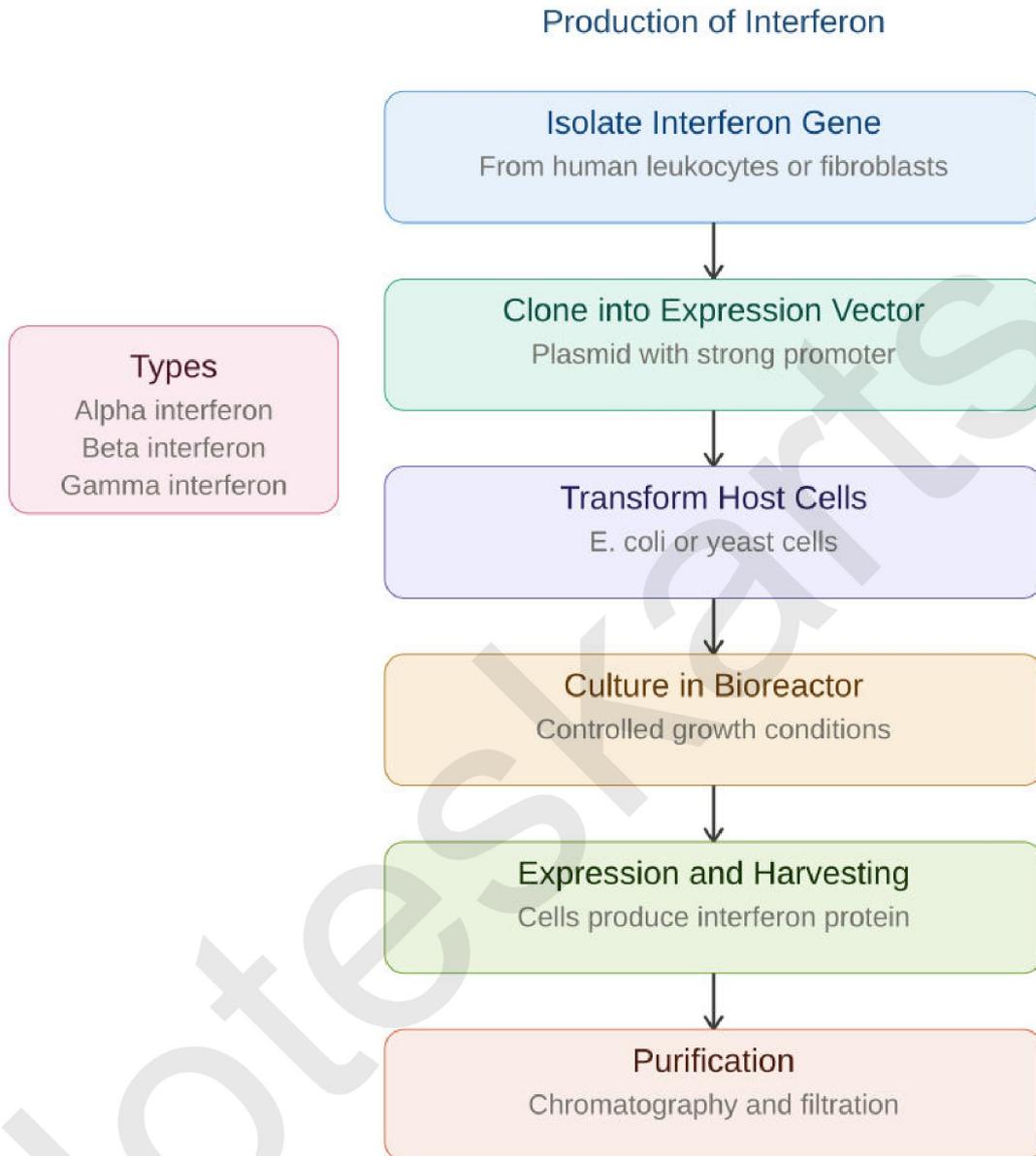
### Medical Applications:

- Treatment of hepatitis B and C
- Treatment of certain cancers (melanoma, leukemia)
- Multiple sclerosis treatment (interferon- $\beta$ )
- Genital warts treatment



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## Production of Interferon using Recombinant DNA Technology



**Figure 4:** Production of Interferon using Recombinant DNA Technology

### Medical Uses of Interferon

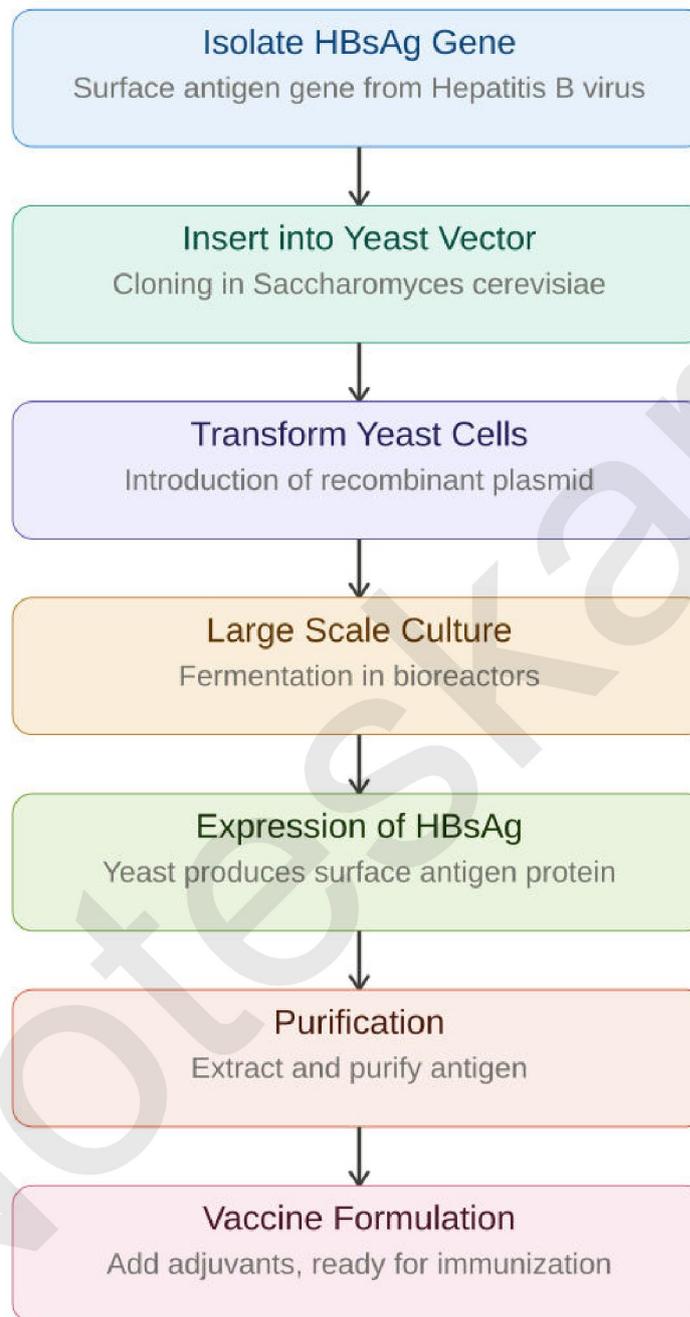
- Treatment of Hepatitis B and C viral infections
- Cancer therapy (certain leukemias and melanomas)
- Treatment of multiple sclerosis

### Production of Hepatitis B Vaccine

The recombinant Hepatitis B vaccine is safer than traditional vaccines because it contains only the surface antigen protein, not the whole virus. This vaccine is produced using yeast cells.



### Production of Hepatitis B Vaccine



Safer than traditional vaccines

**Figure 5:** Production of Hepatitis B Vaccine using Yeast Cells

#### Advantages of Recombinant Vaccine

- No risk of infection (contains no live virus)
- Safer than blood-derived vaccine
- Can be produced in large quantities
- Consistent quality and purity

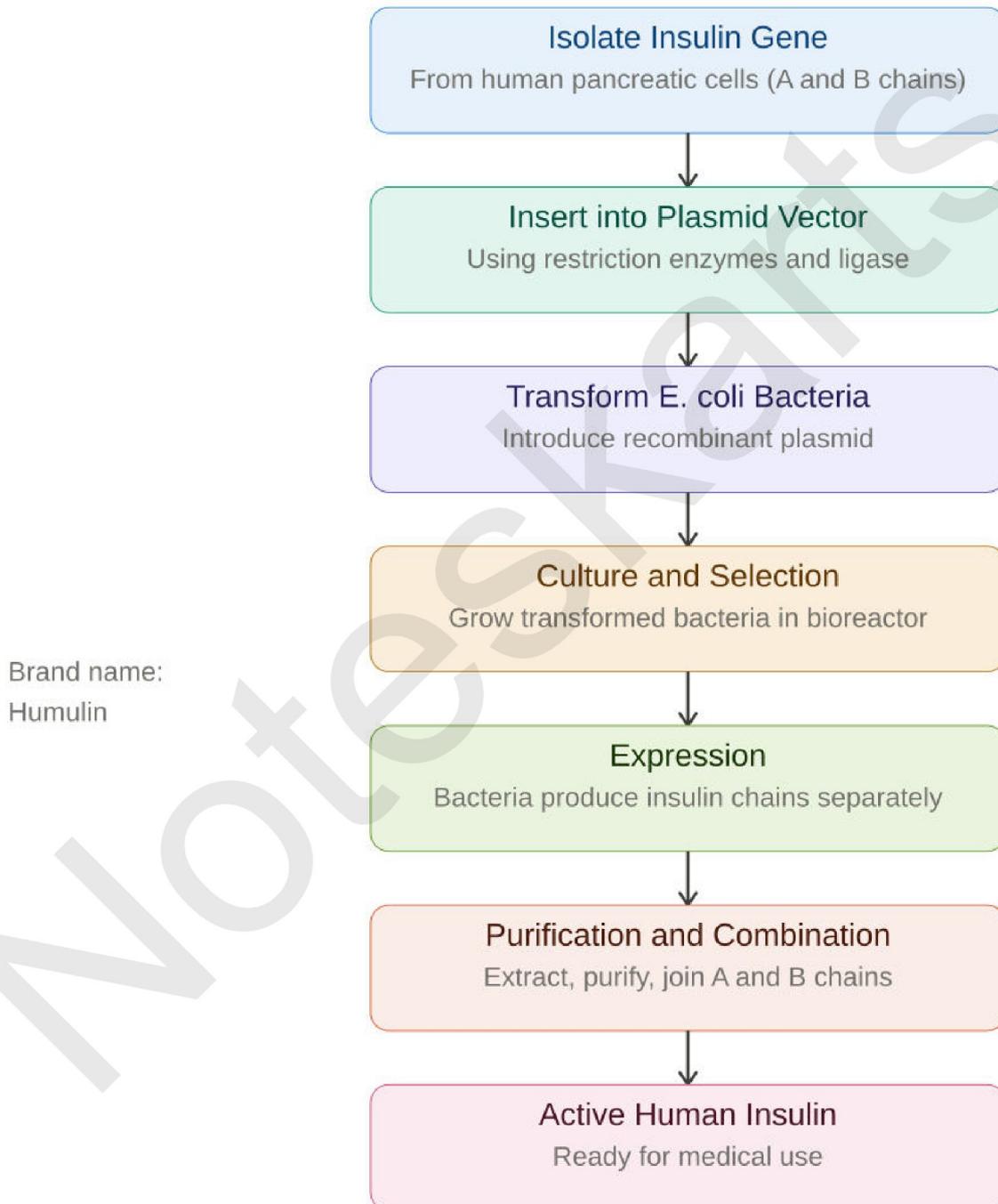


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### Production of Human Insulin (Humulin)

Insulin is essential for regulating blood sugar levels in the body. Before genetic engineering, insulin was extracted from pig and cow pancreas. Now we can produce human insulin using bacteria, which is safer and more effective for patients with diabetes.

#### Production of Human Insulin by rDNA Technology



**Figure 6:** Production of Human Insulin (Humulin) using *E. coli* Bacteria



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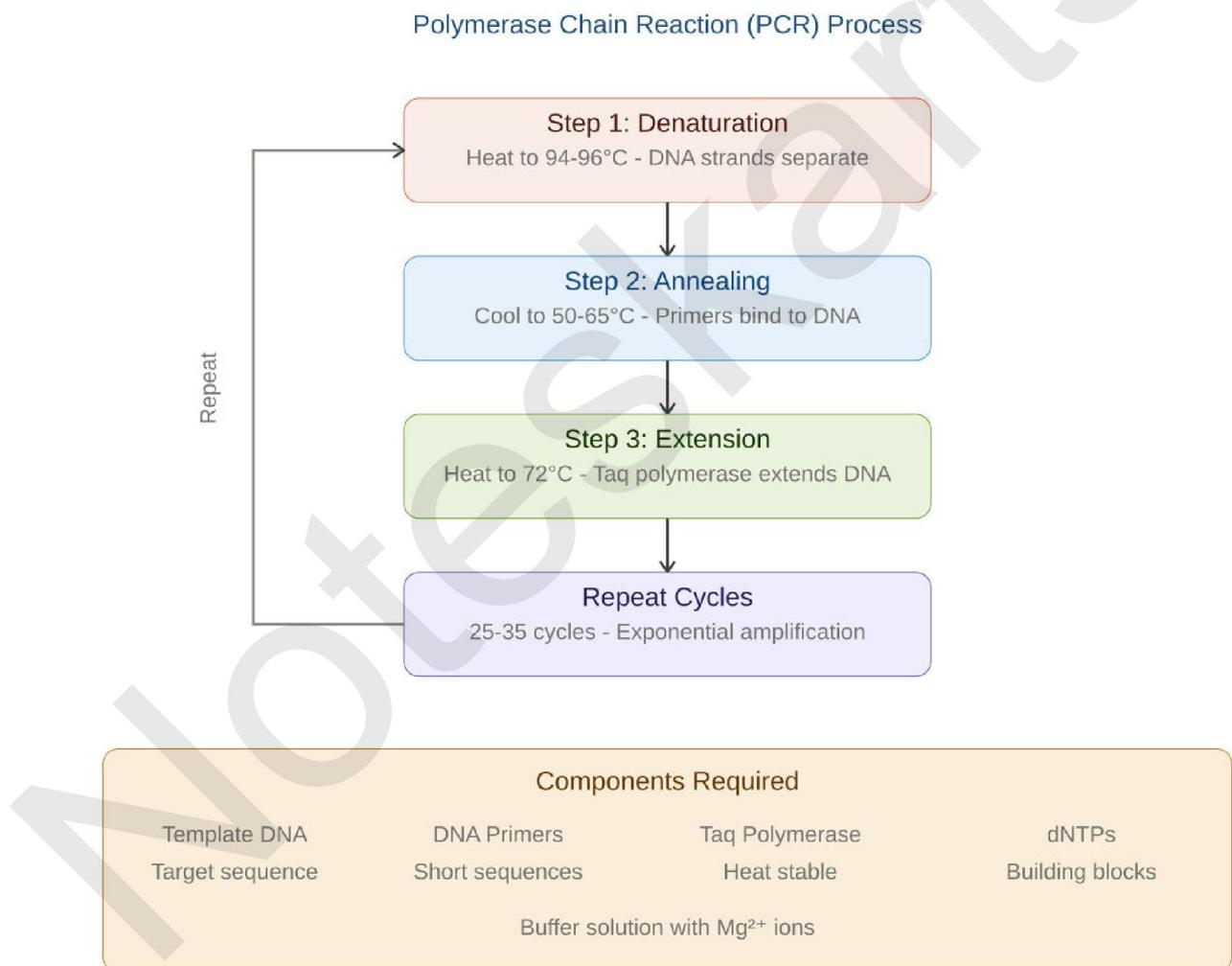
## Points about Insulin Production

- Human insulin has two chains: A chain (21 amino acids) and B chain (30 amino acids)
- Both chains are produced separately in different batches of E. coli bacteria
- The chains are purified and then joined together to form active insulin
- Humulin is identical to human insulin and causes no allergic reactions

## Polymerase Chain Reaction (PCR)

### Introduction to PCR

PCR is a revolutionary technique that allows us to make millions of copies of a specific DNA sequence in just a few hours. Think of it as a molecular photocopier for DNA. This technique was invented by Kary Mullis in 1983, earning him the Nobel Prize.



*Figure 7: The Three Steps of PCR and Required Components*

### The Three Steps of PCR

11. **Denaturation (94-96°C):** High heat separates the double-stranded DNA into two single strands by breaking hydrogen bonds



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12. **Annealing (50-65°C):** Temperature is lowered so primers can bind to their complementary sequences on the DNA template
13. **Extension (72°C):** Taq polymerase enzyme adds nucleotides to extend the primers, creating new DNA strands

### Components Required

- **Template DNA:** The sample containing the target sequence
- **Primers:** Short DNA sequences that mark the start and end of the target region
- **Taq Polymerase:** Heat-stable enzyme from *Thermus aquaticus* bacteria
- **dNTPs:** Building blocks (A, T, G, C nucleotides)
- **Buffer:** Solution with  $Mg^{2+}$  ions needed for enzyme activity

### Applications of PCR

- Medical diagnosis (COVID-19 testing, genetic diseases)
- Forensic science (DNA fingerprinting, crime investigation)
- Paternity testing
- Research (gene cloning, DNA sequencing)
- Agriculture (identifying plant diseases)

